

What is claimed is

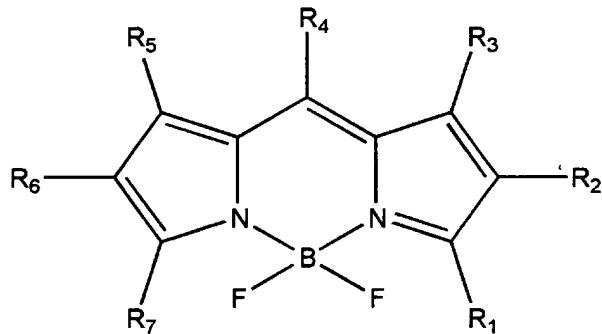
1. A method of labeling poly(amino acids) comprising the steps of:

5 a. separating poly(amino acids) by gel electrophoresis, resulting in separated poly (amino acids);

b. transferring said separated poly(amino acids) to a solid support, resulting in immobilized poly(amino acids) ;

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c. combining said immobilized poly(amino acids) on said solid support with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



TOKYO DATE 05/05/2020 15

wherein each of R¹ through R⁷ are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C₁-C₆ alkyl [carboxylic acid, sulfonic acid, or halogen], aryl, arylethenyl, arylbutadienyl, and heteroaryl [C₁-C₆ alkyl, C₁-C₆ perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl];

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provided that one or more of R¹ through R⁷ is H,

two or more of R¹ through R⁷ is nonhydrogen, and

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only one of R¹ through R⁷ is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

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nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum, between 495 nm and 640 nm;

d. incubating the immobilized poly(amino acids) in the labeling mixture for a sufficient time for the dyes to form a covalent bond with said poly(amino acids), resulting in labeled poly(amino acids).

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2. A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, Rx is a succinimidyl ester of a carboxylic acid.

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3. A method, as claimed in Claim 1, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

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4. A method, as claimed in Claim 3, wherein said solid support is made of materials that are poly(vinylidene difluoride).

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5. A method, as claimed in Claim 1, wherein said poly(amino acids) immobilized on said solid support has a molecular weight of 500 to 200,000 Daltons.

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6. A method, as claimed in Claim 1, wherein said dye is present in the labeling mixture at a concentration of 0.10 micromolar to 10 micromolar.

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7. A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, R¹ is methyl or -L-Rx; R² is H, bromine, or -L-Rx; R³ is H or methyl; R⁴ is H or -L-Rx; R⁵ is H, methyl, or phenyl; R⁶ is H or bromine; and R⁷ is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thiényl; where -L- is -(CH₂)₂- , -(CH₂)₄- , -OCH₂C(O)NH(CH₂)₅- , -(CH₂)₂-C(O)NH(CH₂)₅- ,

$-(CH_2)_2C_6H_4OCH_2C(O)NH(CH_2)_5$;
and Rx is a succinimidyl ester of a carboxylic acid.

8. A method, as claimed in Claim 7, further comprising adding a specific binding pair

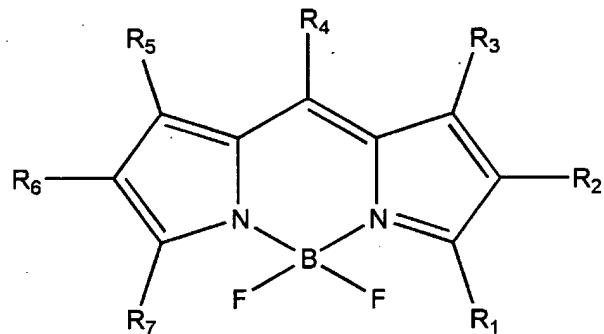
5 member that contains a label and that binds selectively to a target within the immobilized
poly(amino acids) that is its complementary binding pair.

✓ 9. A method of labeling poly(amino acids) bound to aptamers comprising the steps of:

a. incubating immobilized aptamers with poly(amino acids) for a sufficient time to allow
10 said poly(amino acids) to bind to their specific aptamers, resulting in immobilized
poly(amino acids);

b. removing unbound poly(amino acids) that are not immobilized,

15 c. combining said immobilized poly(amino acids) with a labeling mixture that comprises
one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R¹ through R⁷ are independently selected from the group consisting
20 of H, halogen, L-Rx, and substituted or unsubstituted C₁-C₆ alkyl, aryl, arylethenyl,
arylbutadienyl, and heteroaryl;

provided that one or more of R¹ through R⁷ is H,

two or more of R¹ through R⁷ is nonhydrogen, and

only one of R¹ through R⁷ is -L-Rx, where L is a spacer having 1-24 nonhydrogen
25 atoms selected from the group consisting of C, N, O, P, and S and is composed of
any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum
5 between 495 nm and 640 nm;

d. incubating the immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids), resulting in labeled poly(amino acids) that are bound to the
10 aptamers.

10. A method, as claimed in Claim 9, wherein the dipyrrometheneboron difluoride dye's chemically reactive group is a succinimidyl ester of a carboxylic acid.

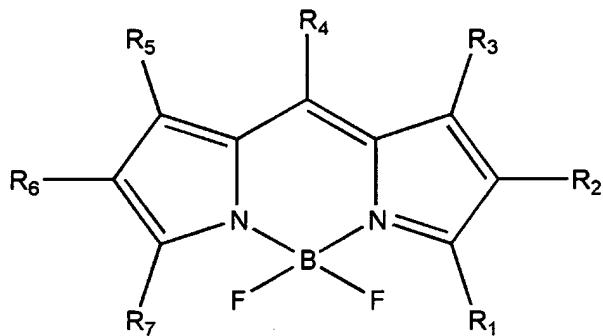
15 11. A method, as claimed in Claim 9, wherein said dipyrrometheneboron difluoride dye is present in the combined labeling mixture at a concentration of 0.10 micromolar to 10 micromolar.

20 12. A method, as claimed in Claim 9, wherein for the dipyrrometheneboron difluoride dye, R¹ is methyl or -L-Rx; R² is H, bromine, or -L-Rx; R³ is H or methyl; R⁴ is H or -L-Rx; R⁵ is H, methyl, or phenyl; R⁶ is H or bromine; and R⁷ is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where -L- is -(CH₂)₂-, -(CH₂)₄-, -OCH₂C(O)NH(CH₂)₅-, -(CH₂)₂-C(O)NH(CH₂)₅-, -(CH)₂C₆H₄OCH₂C(O)NH(CH₂)₅-, and Rx is a succinimidyl ester of a carboxylic acid.
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30 13. A method, as claimed in Claim 12, further comprising adding a specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

14 A method of labeling immobilized poly(amino acids) in an array comprising the steps
of:

- 5 a. combining an array of immobilized poly(amino acids) with a labeling mixture that
comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of
the formula



10 wherein each of R¹ through R⁷ are independently selected from the group consisting
of H, halogen, L-Rx, and substituted or unsubstituted C₁-C₆ alkyl, aryl, arylethenyl,
arylbutadienyl, and heteroaryl;

15 provided that one or more of R¹ through R⁷ is H,

 two or more of R¹ through R⁷ is nonhydrogen, and

 only one of R¹ through R⁷ is -L-Rx, where L is a spacer having 1-24 nonhydrogen
atoms selected from the group consisting of C, N, O, P, and S and is composed of
any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–
nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur
bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds; and Rx is a
reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;

20 such that the dipyrrometheneboron difluoride dye has an absorption maximum
between 495 nm and 640 nm;

- 25 b. incubating said array with the labeling mixture for a sufficient time to form a covalent
bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino
acids), resulting in the array of poly(amino acids) being labeled.

15. A method, as claimed in Claim 14, wherein for the dipyrrometheneboron difluoride dye, Rx is a succinimidyl ester of a carboxylic acid.

16. A method, as claimed in Claim 14, wherein said dipyrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0.10 micromolar - 10 micromolar.

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17. A method, as claimed in Claim 14, wherein for the dipyrrometheneboron difluoride dyes, R¹ is methyl or -L-Rx; R² is H, bromine, or -L-Rx; R³ is H or methyl; R⁴ is H or -L-Rx; R⁵ is H, methyl, or phenyl; R⁶ is H or bromine; and R⁷ is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutatdienyl pyrrolyl, or thienyl;

10 where -L- is -(CH₂)₂-, -(CH₂)₄-, -OCH₂C(O)NH(CH₂)₅-, -(CH₂)₂-C(O)NH(CH₂)₅-,

(CH₂)₂C₆H₄OCH₂C(O)NH(CH₂)₅;

and Rx is a succinimidyl ester of a carboxylic acid.

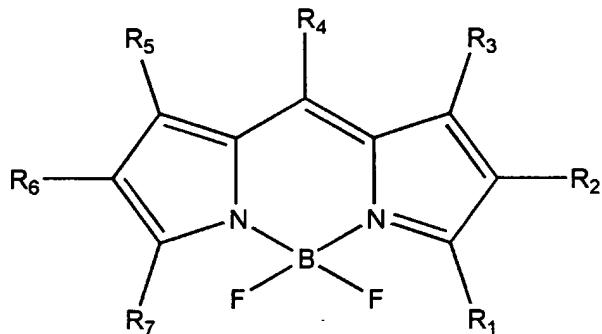
15 18. A method, as claimed in Claim 17, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

20 19. A method, as claimed in Claim 14, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

20 A method of detecting poly(amino acids) comprising the steps of:

a. combining poly(amino acids) immobilized on a solid support; with a labeling mixture

25 that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula



wherein each of R¹ through R⁷ are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C₁-C₆ alkyl, aryl, arylethenyl, 5
arylbutadienyl, and heteroaryl;

provided that one or more of R¹ through R⁷ is H,

two or more of R¹ through R⁷ is nonhydrogen, and

only one of R¹ through R⁷ is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

b. incubating said immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrrometheneboron difluoride dye and said immobilized poly(amino acids) resulting in labeled poly(amino acids);

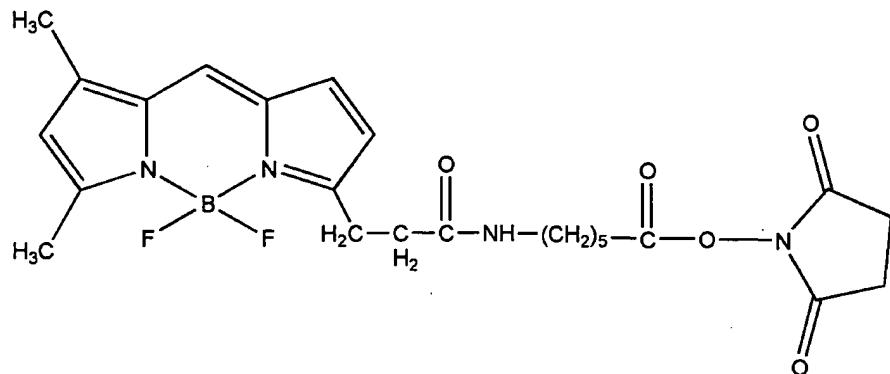
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c. removing unbound dipyrrrometheneboron difluoride dyes ;

d. illuminating said labeled poly(amino acids) to yield a fluorescent optical response to detect the corresponding labeled poly(amino acids).

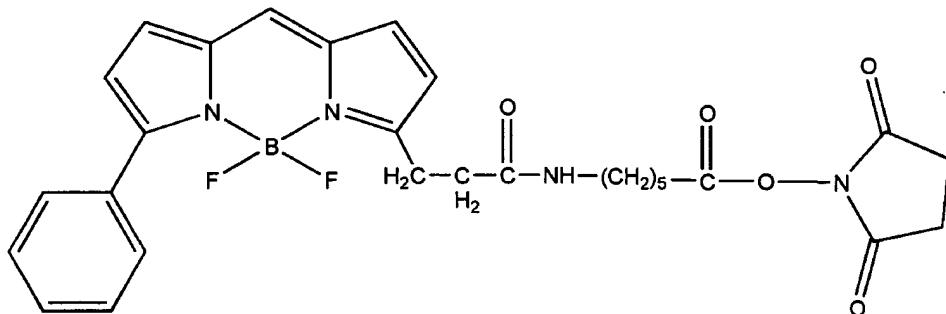
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21. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



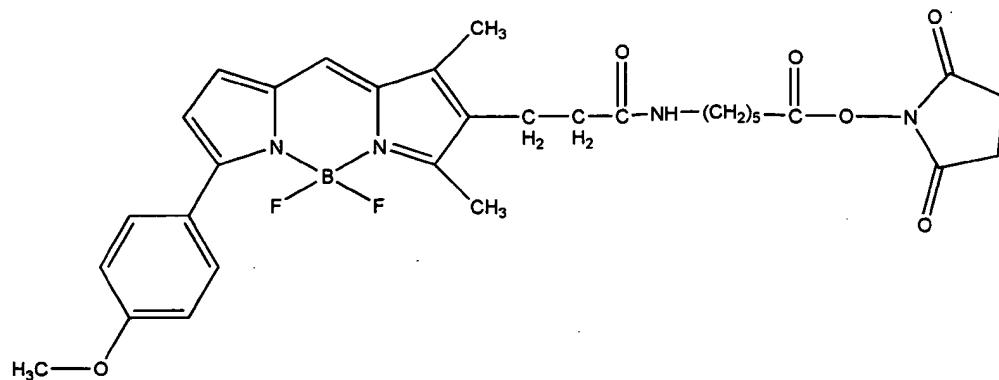
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22. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

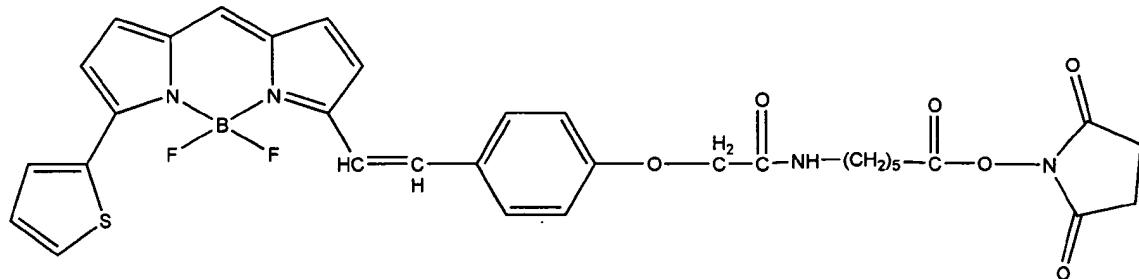


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23. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

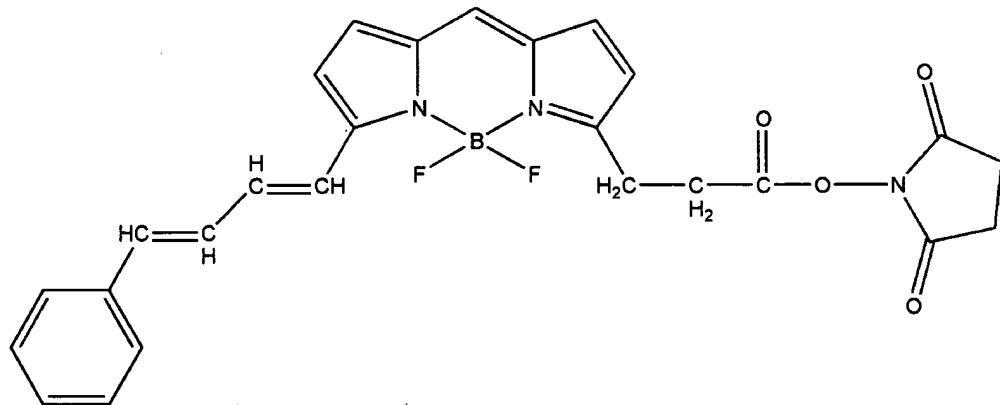


24. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



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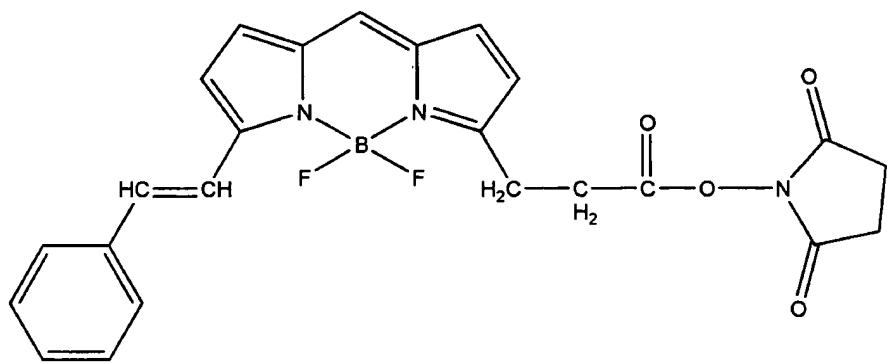
25. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



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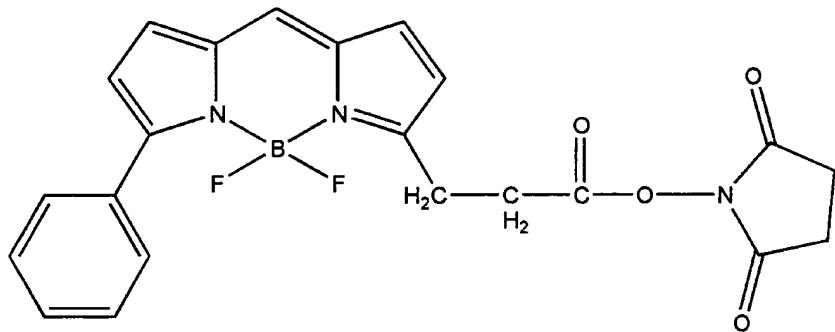
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26. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



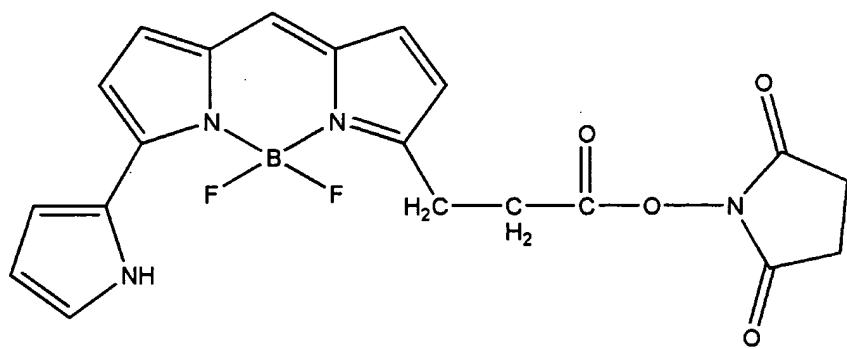
27. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

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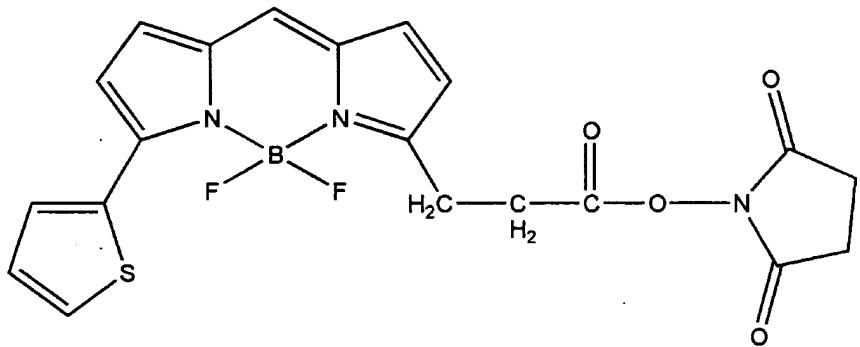


28. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

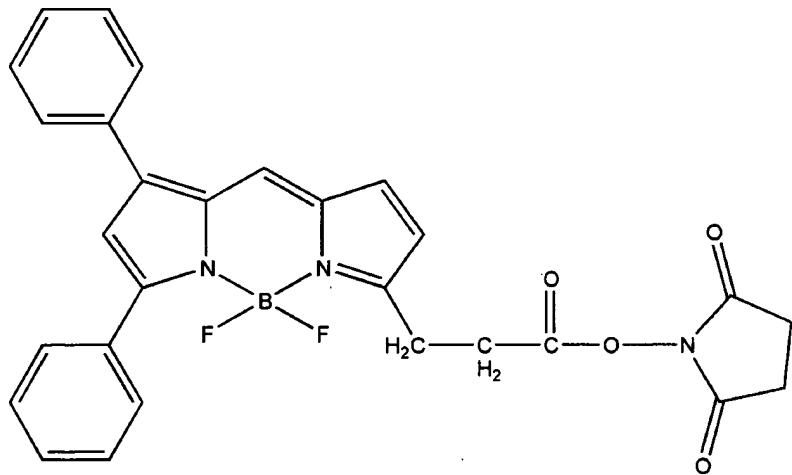
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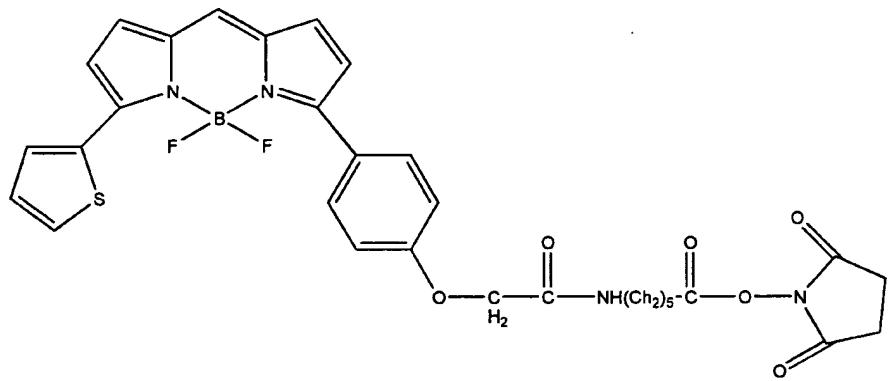
29. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



30. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has
5 the formula:



10 31. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has
the formula:



32. A method, as claimed in Claim 20, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

5 33. A method, as claimed in Claim 32, wherein said solid support is made of materials that
are poly(vinylidene difluoride).

10 34. A method, as claimed in Claim 20, wherein said poly(amino acids) on said solid support each have a molecular weight of between 500 Daltons and 200,000 Daltons.

15 35. A method, as claimed in Claim 20, wherein for said dipyrrrometheneboron difluoride dye R¹ is methyl or -L-Rx; R² is H, bromine, or -L-Rx; R³ is H or methyl; R⁴ is H or -L-Rx; R⁵ is H, methyl, or phenyl; R⁶ is H or bromine; and R⁷ is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutatdienyl pyrrolyl, or thienyl; where -L- is -(CH₂)₂-, -(CH₂)₄-, -OCH₂C(O)NH(CH₂)₅-, -(CH₂)₂-C(O)NH(CH₂)₅-, -(CH)₂C₆H₄OCH₂C(O)NH(CH₂)₅-, and Rx is a succinimidyl ester of a carboxylic acid..

20 36. A method, as claimed in Claim 35, wherein said dipyrrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0.10 micromolar to 10 micromolar, and

wherein said labeled poly(amino acids) are illuminated for five seconds or less.

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37. A method, as claimed in Claim 20, further comprising adding a specific binding pair member that selectively binds to a target within said immobilized poly(amino acids) that is its complementary binding pair.

5 38. A method, as claimed in Claim 37, where said specific binding pair member contains a label that is an enzyme or a hapten.

39. A method, as claimed in Claim 37, where said specific binding pair member contains a label that is a fluorophore.

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40. A method, as claimed in Claim 37, further comprising:
adding a secondary complementary binding pair member that contains a label and that selectively binds to the specific binding pair member.

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41. A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is an enzyme.

42. A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is a fluorescent dye.

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43. A method, as claimed in Claim 41, wherein said enzyme is a peroxidase or a phosphatase.

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44. A method, as claimed in Claim 43, wherein said peroxidase is horseradish peroxidase.

45. A method, as claimed in Claim 43 wherein said phosphatase is alkaline phosphatase.

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46. A method, as claimed in Claim 41, wherein said enzyme is capable utilizing a fluorogenic substrate to generate a detectable optical response.

TECHNOLOGY

47. A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a fluorescent tyramide.

48. A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is a quinazolinone phosphate.

49. A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is 9H-(1,3-dichloro-9,9-dimethylacridin- 2-one-7-yl) phosphate.

10 50. A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a polyfluorinated xanthene.

15 51. A method, as claimed in Claim 40, wherein said secondary complimentary binding pair is an antibody or an antibody fragment.

52. A method, as claimed in Claim 39, wherein said complementary specific binding pair member is a lectin.

20 53. A method, as claimed in Claim 39, wherein said specific binding pair member is biotin-binding protein that contains a label.

54. A method, as claimed in Claim 53, wherein said biotin-binding protein is streptavidin.

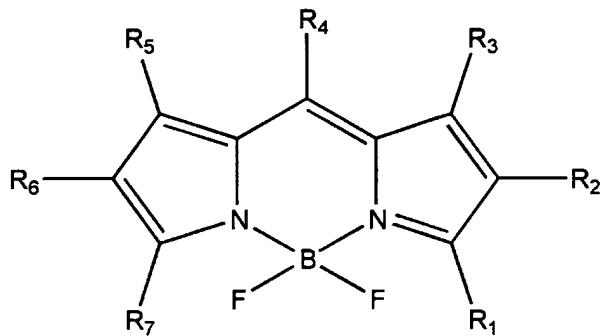
55. A method, as claimed in Claim 53, wherein said biotin-binding protein is NeutrAvidin.

25 56. A method, as claimed in Claim 37, wherein said specific binding pair member is an antibody or antibody fragment, an aptamer, a lectin, or a biotin-binding protein.

57. A kit for detection of poly(amino acids) immobilized on a solid surface, said kit comprising:

30 a. a dipyrrometheneboron difluoride dye of the formula:

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wherein each of R¹ through R⁷ are independently selected from the group consisting
5 of H, halogen, L-Rx, and substituted or unsubstituted C₁-C₆ alkyl, aryl, arylethenyl,
arylbutenyl, and heteroaryl;

provided that one or more of R¹ through R⁷ is H,

two or more of R¹ through R⁷ is nonhydrogen, and

only one of R¹ through R⁷ is -L-Rx, where L is a spacer having 1-24 nonhydrogen
10 atoms selected from the group consisting of C, N, O, P, and S and is composed of
any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–
nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur
bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds; and Rx is a
reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;
such that the dipyrrrometheneboron difluoride dye has an absorption maximum
15 between 495 nm and 640 nm;

b. a specific binding pair member that contains a label and that selectively binds to a target
that is its complementary binding pair.

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58. A kit, as claimed in Claim 57, wherein the specific binding pair member contains a
label that is an enzyme; wherein said enzyme is capable utilizing a fluorogenic substrate to
generate a detectable optical response, said kit further comprising the fluorogenic substrate.

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59. A kit, as claimed in Claim 57, wherein said specific binding pair member is an antibody
or antibody fragment.

60. A kit, as claimed in Claim 57, wherein the specific binding pair member contains a label that is a fluorescent dye.

5 61. A kit, as claimed in Claim 57, wherein said specific binding pair member is a biotin-binding protein.

62. A kit, as claimed in Claim 61, wherein said biotin-binding protein is avidin, Neutravidin or streptavidin.

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63. A kit, as claimed in Claim 58, wherein said label is an enzyme that is a peroxidase or a phosphatase.

64. A kit, as claimed in Claim 63, wherein said peroxidase is horseradish peroxidase.

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65. A kit, as claimed in Claim 64, wherein said fluorogenic substrate peroxidase substrate that is a fluorescent tyramide.

66. A kit, as claimed in Claim 63, wherein said phosphatase is alkaline phosphatase.

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67. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is a 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate.

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68. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is a 2-(5'-chloro- 2'-phosphoryloxyphenyl)-6-chloro- 4(3H)-quinazolinone.

69. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is ELF 39 reagent.

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70. A kit, as claimed in Claim 58, wherein

for the dipyrrometheneboron difluoride dye, R¹ is methyl or -L-Rx; R² is H, bromine, or -L-Rx; R³ is H or methyl; R⁴ is H or -L-Rx; R⁵ is H, methyl, or phenyl; R⁶ is H or bromine; and R⁷ is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where -L- is -(CH₂)₂- , -(CH₂)₄- , -OCH₂C(O)NH(CH₂)₅- , -(CH₂)₂-C(O)NH(CH₂)₅- ,

5 -(CH)₂C₆H₄OCH₂C(O)NH(CH₂)₅- ;

and Rx is a succinimidyl ester of a carboxylic acid;

the specific binding pair member is an antibody or a streptavidin that contains a label that is an alkaline phosphatase and the fluorogenic substrate is a 9H-(1,3-dichloro-9,9-

10 dimethylacridin-2-one-7-yl) phosphate, a 2-(5'-chloro- 2'-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone, or ELF 39 reagent.

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